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Scientific Discussion

Editors' note: *Deoxyglucose has been widely used as a tracer to measure local brain glucose utilization. Several authors have questioned the stability of deoxyglucose-6-phosphate in the brain. An article which appeared in this* Journal *[Nelson et al.* J. Neurochem. ***46,*** *905–919 (1986)] addressed this issue. The submission by Hawkins and Miller which follows is a critique of that paper and the deoxyglucose method. Nelson et al. were given the opportunity to respond. The editors believe that the neurochemical community will find it of interest to compare these two papers in this extensive special section.*

Deoxyglucose-6-Phosphate Stability In Vivo and the Deoxyglucose Method

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Some of the assumptions underlying the 2-deoxy-D-glucose (DG) method for measuring the rate of cerebral glucose metabolism (CMRglc) have come into question (Fox, 1984; Bachelard, 1985; Cunningham and Cremer, 1985). An important point is whether much label is lost from brain during the experimental period by catabolism of 2-deoxy-D-glucose-6-phosphate (DG-6-P). This issue was the subject of an article published recently in this Journal by Nelson et al. (1986*a*). Because of the importance of the subject, we have reviewed the data concerning DG-6-P catabolism in vivo.

The goal of the DG method is to measure the rate of glucose utilization in cerebral structures using quantitative autoradiography. The essential requirement is a tracer of glucose whose label remains in brain at the site of its metabolism. Sokoloff et al. (1977) chose DG, a glucose analog that competes with glucose for transport and phosphorylation, because its phosphorylated product, DG-6-P, was thought not to be metabolized or dephosphorylated. However, glucose-6-phosphatase (G-6-Pase), an enzyme that is found in low activities in brain as well as many other tissues (Nordlie, 1971), is capable of hydrolyzing both glucose-6-phosphate (G-6-P) and DG-6-P with almost equal facility (Anchors and Karnovsky, 1975). (There are other intracellular phosphatases that could conceivably react with DG-6-P, but G-6-Pase seems to be the most likely to do so.) Figure 1 shows what would happen to brain DG-6-P

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Abbreviations used: CMRglc, rate of cerebral glucose metabolism; DG, 2-deoxy-D-glucose; DG-6-P, 2-deoxy-D-glucose-6-phosphate; FDG, fluoro-2-deoxy-D-glucose; G-6-P, glucose-6-phosphate; G-6-Pase, glucose-6-phosphatase.

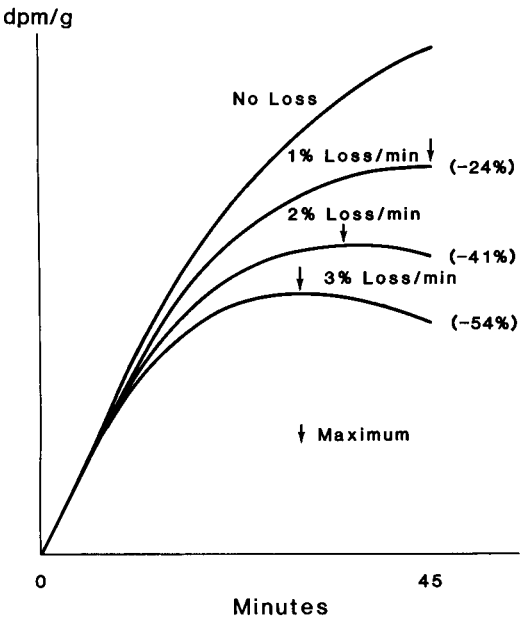

FIG. 1. Accumulation and loss of DG-6-P in the presence of G-6-Pase. The predicted brain content of DG-6-P as a function of time after a single intravenous injection of DG is shown for various phosphatase activities. The numbers in parentheses indicate the cumulative loss of DG-6-P at 45 min. The arrows indicate the maximum brain DG-6-P content. The predicted time course of DG-6-P accumulation by brain was calculated assuming single pools of DG, DG-6-P in brain and a first-order hydrolysis of DG-6-P to DG. Thus $d(DG_b)/dt = k_1(DG_p) - (k_2 + k_3)(DG_b) + k_4(DG\text{-}6\text{-}P)$ and $d(DG\text{-}6\text{-}P)/dt = k_3(DG_b) - k_4(DG\text{-}6\text{-}P)$, where the subscripts p and b refer to plasma and brain, respectively. The values for k_1, k_2, and k_3 were those reported for gray matter (Sokoloff et al. 1977) and k_4 was permitted to change from 0 (no loss) to 0.03/min (3%/min). The plasma input function was taken to be: $DG = e^{-0.5t} + e^{-0.05t}$. The brain curves were calculated by numerical integration over 45 min.

at various rates of hydrolysis after a single injection of DG. If DG-6-P were removed at the rate of only 1%/min steadily from the beginning of an experiment the net loss would be about 24% at 45 min; at 3%/min the loss would be about 54%. Even small amounts of G-6-Pase could seriously affect the retention of DG-6-P by brain during the required 45-min period. The important questions are: (1) Does G-6-Pase exist in brain and if so where and how active is it? (2) Does G-6-Pase react with DG-6-P in vivo? (3) If G-6-Pase is active, does it react with DG-6-P throughout the experimental period? (4) Is G-6-Pase distributed homogeneously throughout brain? (5) Does G-6-Pase activity vary under different conditions. These questions will be addressed below.

DOES G-6-PASE EXIST IN BRAIN AND, IF SO, WHERE AND HOW ACTIVE IS IT?

G-6-Pase is a multifunctional enzyme that acts as a phosphotransferase or as a phosphohydrolase (Nordlie, 1975). Biochemical data suggest that cerebral G-6-Pase functions primarily as a phosphohydrolase in vivo. Although the natural substrate is G-6-P, G-6-Pase reacts nearly as well with DG-6-P (Anchors et al., 1977; Hawkins and Miller, 1978). The first complete purification of G-6-Pase was from brain tissue (Anchors and Karnovsky, 1975); purification of G-6-Pase from liver, the tissue normally considered to contain the greatest activity, has not yet been possible (Fishman and Karnovsky, 1986). Brain G-6-Pase activity has been identified cytochemically in neurons, astrocytes, and oligodendroglia as well as almost every other brain cell type (Rosen, 1970; Stephens and Sandborn, 1976; Broadwell and Cataldo, 1983, 1984; Cataldo and Broadwell, 1986). Anchors and Karnovsky (1975) found no large differences in activity across brain regions, but Stephens and Sandborn (1976) found the cerebellum to contain 36% more activity than the cerebrum.

G-6-Pase activity appears variable. In studies of rat brain metabolism during slow-wave sleep, the phosphate moiety of G-6-Pase turned over about six times more rapidly during sleep than wakefulness (Anchors and Karnovsky, 1975) and enzyme activity was increased in slow-wave sleep (Karnovsky et al., 1980). Cataldo and Broadwell (1986) found changes in G-6-Pase activity within certain cell types during "stressed states" (e.g., salt-stress, short-term starvation, and surgery).

Intracellularly, G-6-Pase activity is associated with the lumen of the nuclear envelope, endoplasmic reticulum, lamellar bodies, and the cis saccule of the Golgi complex (Anchors and Karnovsky, 1975; Stephens and Sandborn, 1976; Broadwell and Cataldo, 1983; Cataldo and Broadwell, 1986). G-6-Pase activity in axons is minor under normal conditions compared to the cell bodies and dendrites (Broadwell and Cataldo, 1983, 1984) unless the axons are injured (Cataldo and Broadwell, 1986). The most prevalent position of G-6-Pase is on the inside of the endoplasmic reticulum; therefore, G-6-Pase is separated by a membrane from its substrate G-6-P (or DG-6-P), which is formed on the cytoplasmic side (Leskes et al., 1971; Arion et al., 1975; Ballas and Arion, 1977; Broadwell and Cataldo, 1983). In liver, a translocase is believed to mediate the movement of G-6-P from the cytoplasm to the lumen of the endoplasmic reticulum wherein resides G-6-Pase; a similar translocase has yet to be identified in brain. For this reason the mechanism by which the substrate is delivered across the endoplasmic reticular membrane remains to be clarified (Fishman and Karnovsky, 1986). In contrast to the endoplasmic reticulum the nucleus has a fenestrated membrane that allows the passage of small molecules. Thus nuclear G-6-Pase is available to cytoplasmically synthesized G-6-P and DG-6-P.

The activity of G-6-Pase has been measured in a number of laboratories. The results show a range of V_{max} values from 3 $\mu mol \cdot min^{-1} \cdot hg^{-1}$ (Sokoloff et al., 1977) to 160 $\mu mol \cdot min^{-1} \cdot hg^{-1}$ (Prasannan and Subrahamanyam, 1968) as summarized in Table 1.

TABLE 1. *Activity of G-6-Pase in rat brain*

Source	V_{max} (μmol · hg^{-1} · min^{-1})
Anchors et al. (1977)	48 *p*
Colilla et al. (1975)	19 *t*[a]
Fishman and Karnovsky (1986)	33 *p*
Hers (1957)	9[a]
Hawkins and Miller (1978)	46
Nordlie and Arion (1965)	84 *t*
Prasannan and Subrahamanyam (1968)	160[a]
Sokoloff et al. (1977)	3[a]
Stephens and Sandborn (1976)	82 *p*[a]

Original values given in reference to protein were converted assuming 100 mg protein/g fresh weight (*p*). Values measured at temperatures lower than 38°C were adjusted to 38°C assuming a Q_{10} of 2 (*t*). The value of Stephens and Sandborn (1976) is the average reported by them for the cerebrum (70) and the cerebellum (95). Full activity of G-6-Pase from broken-cell preparations is realized only after disruption of membrane structures by sonication or by the use of detergents (Karnovsky et al., 1982).

[a] Experiments where the membranes were not disrupted.

G-6-Pase is greatly affected by tissue preparation and assay conditions and this may explain the wide range in activities found (Baginski et al., 1967; Karnovsky et al., 1982). Although it has been calculated that there is enough G-6-Pase to hydrolyze about 6% of the brain's DG-6-P content per minute (Hawkins and Miller, 1978), it is not possible to extrapolate measurements made in vitro to activity in vivo with complete accuracy because local conditions are not known with certainty. Also it is not known whether other phosphatases react with DG-6-P. Nevertheless the data do show that brain G-6-Pase exists with sufficient potential activity to cause concern and that G-6-P reacts nearly as well as DG-6-P as with G-6-P.

IS DG-6-P LOST AT AN APPRECIABLE RATE IN VIVO?

Although enough G-6-Pase activity exists in brain to hydrolyze substantial quantities of DG-6-P, the

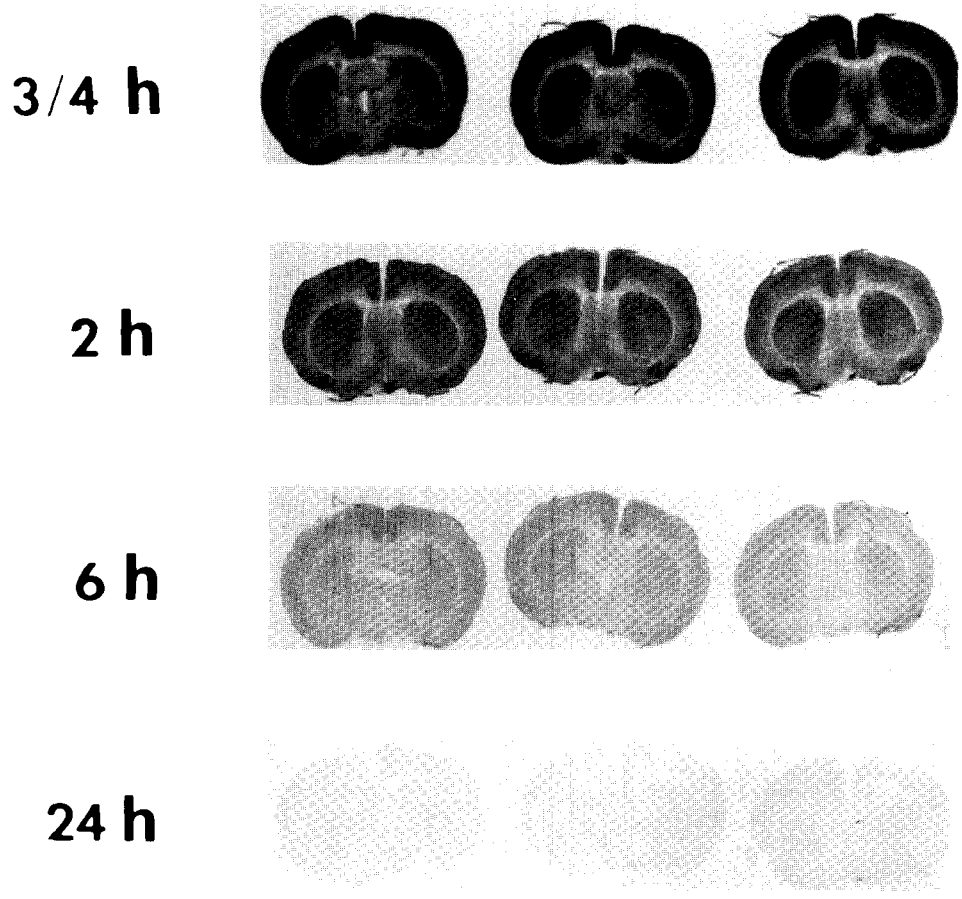

FIG. 2. Brain loses DG as time passes. Four overnight fasted rats, matched for age and weight, were given a single intravenous injection of [^{14}C]DG (46 μCi) and killed at 45 min and 2, 6, and 24 h. In comparison to images made at 45 min (1,000 dpm/g normalized value) the images are much lighter at 2 h (520 dpm/g), at 6 h they are very pale (230 dpm/g), and at 24 h they are almost invisible (70 dpm/g). The values reported by Sokoloff et al. (1977) were made from autoradiographs 17–24 h after administration of a dose of DG by which time >90% of the radioactivity had been lost. They thought the only metabolite of DG was DG-6-P, but it is now known that other compounds were present and the half-lives observed between 17 and 24 h reflected the turnover of these compounds rather than DG-6-P (Hawkins and Miller, 1978; see note 2 under References in Nelson et al., 1986*b*).

important issue is whether hydrolysis occurs. Sokoloff et al. (1977) reported the half life of DG-6-P to be 7.7 and 9.7 h for gray and white tissue respectively, corresponding to first-order rate constants of 0.15 and 0.12% loss per minute. If these figures were accurate it may be appreciated from Fig. 1 that DG-6-P loss would be almost inconsequential over the 45-min experimental period required by the DG method. These estimates were based on measures of residual brain radioactivity made by quantitative autoradiography 17–24 h after injection of [^{14}C]DG. These experiments had two flaws. First, after 17 h the brains had lost almost all their radioactivity (Fig. 2). Second, most of the residual activity at that late time was not in the form of DG-6-P.

Shortly after Sokoloff et al. (1977) reported their observations, articles were published describing DG-6-P loss in rats (Hawkins and Miller, 1978) and fluoroDG-6-P (FDG-6-P) loss in humans (Phelps et al., 1979). The experiments on humans, using positron emission tomography (PET), were especially important because they extended the three-compartment model for CMRglc to include the hydrolysis of FDG-6-P to FDG. Phelps and associates found that although loss of FDG-6-P was not fast it was sufficient to cause significant errors depending on how long after injection CMRglc was measured. They determined a loss constant (k_4) by repeated scanning of patients over a period of hours and fitting the data to obtain the various rate constants including an estimate of k_4. In this way an estimate of loss for each individual patient was made and applied throughout the experimental period. Over the course of several studies a range of k_4 values from 0.58 ± 0.17 (SD) in normal humans to 1.16 ± 0.16 in patients with brain disease was found (Phelps et al., 1979; Hawkins et al., 1981; Kato et al., 1984).

Subsequently several other laboratories measured the stability of DG-6-P by one means or another in a variety of species. A summary of the results (Table 2) shows that DG-6-P loss occurs at rates greater than suggested by early work.

TABLE 2. *Loss of DG-6-P in vivo*

Source	Species	Rate constant (%/min)	Half-life (min)
Deuel et al. (1985)	Rat	0.57–0.77	90–122
Hawkins and Miller (1978)	Rat	0.7–3.5	20–100
Huang and Veech (1985)	Rat	3.00–5.4	13–23
Karnovsky et al. (1980)	Rat (sleeping)	2.4	29
Sokoloff et al. (1977)	Rat	0.12–0.15	462–582
Kato et al. (1984)	Dog	0.96	72
Pelligrino et al. (1987)	Goat (awake)	1.18	59
Pelligrino et al. (1987)	Goat (anesthetized)	1.86	37
Hawkins et al. (1981)	Human	0.8	87
Kato et al. (1984)	Human	1.3	53
Phelps et al. (1979)	Human	0.68	102

The relationship between half-life ($t_{1/2}$) and the rate constant of loss (K_{loss}) is: $t_{1/2} = \ln 2/K_{loss}$. Deuel et al. (1985) observed that DG-6-P in vivo reached a peak between 30 and 50 min. This is compatible with a loss constant between 1 and 3%/min (see Fig. 1). The values Deuel et al. reported (shown above) were based on the rate of decline of brain DG-6-P content after its peak. They are underestimates because the continued phosphorylation of DG that occurred during that period was not taken into account. The values calculated from Karnovsky et al. (1980) refer to the increased rate of brain DG-6-P loss in sleeping rats in relation to controls; the absolute rate must have been greater.

WHEN DOES LOSS OF DG-6-P BEGIN?

Nelson et al. (1986*a*) debate some of the evidence, but they accept that DG-6-P disappears considerably faster than originally reported by Sokoloff et al. (1977), and they recognize that the early experiments probably measured the turnover of other metabolites than DG-6-P (see note 2 under references in Nelson et al., 1986*b*). However, they argue that loss does not take place during the first 45 min because, as mentioned above, the enzyme and substrate are in different cellular compartments; G-6-Pase is located in the endoplasmic reticulum and DG-6-P is formed in the cytoplasm (Sokoloff, 1982; Nelson et al., 1986*a,b*). Nelson et al. (1986*a*) cite as evidence for their hypothesis the work of Fishman and Karnovsky (1986), who found that brain G-6-Pase system is different from that of liver. In liver a translocase is thought to be important in mediating the movement of G-6-P from the cytosol to the endoplasmic reticulum; in brain no translocase can be found.[1] Whereas the absence of an identifiable translocase in brain is of interest, it does not prove that a delay in dephosphorylation occurs; it only provides an explanation of why the phosphorylation of DG-6-P might be delayed if the endoplasmic reticulum were the only site of dephosphorylation. Also the hypothesis fails to consider the effect of nuclear G-6-Pase which is not separated from the cytoplasm by an impermeable barrier.

Pelligrino et al. (1987) dispute the hypothesis of delayed dephosphorylation. They note that in a passive system the rate of DG-6-P movement into the endoplasmic reticulum will be a function of the cytoplasm-to-endoplasmic reticulum concentration gradient and the permeability of the endoplasmic reticulum, and by 10 min this gradient has already reached 50–80% of its maximal value. For no DG-6-P loss to

[1] The data of Fishman and Karnovsky (1986) cannot be regarded as definitive; they found about half the G-6-Pase activity to be latent, but about half was active. It should be kept in mind that after homogenization the membranes of the endoplasmic reticulum are disrupted and roll up into small vesicles. Whether these artificial vesicles accurately reflect normal intracellular function is a matter of speculation.

occur during the first 45 min would require a sudden increase in permeability during the 45–60 min period; such an occurrence is without precedent. Furthermore, Pelligrino et al. (1987) point out that the delay-hypothesis predicts that accelerated rates of loss occur after 45 min and this is contrary to experimental observations.

Although theoretical arguments are useful in formulating ideas, actual measurements have been made in vivo that suggest that DG-6-P hydrolysis may begin much earlier than 45 min. In addition to the experiments summarized below, in which determinations were made on brain, there are other relevant experiments on arteriovenous differences contained in the footnote below.[2]

Deuel et al. (1985) used both nuclear magnetic resonance and gas chromatography to follow brain DG-6-P after a single intravenous injection of DG. They found that DG-6-P peaked at 30–50 min and fell thereafter. As shown in Fig. 1 this behavior is predicted when a phosphatase is continuously hydrolyzing DG-6-P at a constant rate between 1 and 3%/min. Nelson et al. (1986*a*) interpret the data as showing that after 45 min "effects of DG-6-Pase activity begin to appear which become progressively more profound with increasing time." The data of Deuel et al. (1985) confirm that DG-6-P is not stable in vivo, but they do not clearly discern between the possible interpretations. On the other hand the DG-6-P decay curves measured by Deuel et al. (1985) were linear and showed no evidence of a progresive increase in the rate of hydrolysis.

Huang and Veech (1985) tested DG-6-P stability directly in two experiments. In the first [^{14}C]DG was injected into the carotid artery and in the other it was given intravenously. They determined brain [^{14}C]DG-6-P content during the next 50 min. We reviewed the data of Huang and Veech with the Statistical Consulting Center of The Pennsylvania State University and came to the following conclusions. In the intracarotid experiment [^{14}C]DG-6-P reached a peak at about 10–12 min and then fell thereafter at a rate of 1.2%/min. With regard to the second experiment (intravenous injection) the fit to the brain [^{14}C]DG-6-P data given by Huang and Veech, which considered both phosphorylation and dephosphorylation (5%/min), was better than any possible value of phosphorylation alone. Thus, the data of Huang and Veech suggest that dephosphorylation, at rates between 1.2 and 5%/min, may begin as early as 10–12 min.

Hawkins et al. (1988) demonstrated DG-6-P loss between 20 and 45 min using a "glucose-chase" experiment. A group of male rats matched by age and weight were prepared with catheters, restrained, and allowed to recover for a typical DG experiment. All rats were given a single identical injection of [^{14}C]DG. Half the rats were killed at 20 min; the remainder were given a large intravenous injection of nonradioactive glucose to curtail further phosphorylation of DG by brain. The glucose-injected rats were killed at 45 min. Measurement of DG-6-P in extracts of freeze-blown brains showed the rats killed at 45 min to have 19% less DG-6-P, thereby demonstrating an appreciable loss within the experimental period normally used for the DG method. It could be argued that the conditions of the experiment activated otherwise latent DG-6-Pase activity. This seems, on the face of it, a somewhat unlikely possibility. If true, however, it would suggest that DG-6-Pase activity is subject to acute variation under different conditions.

The accumulation of DG-6-P was studied in goats by Pelligrino et al. (1987). They measured DG and DG-6-P in brain biopsies using a sequential sampling technique. This procedure, in which the tissue precursor specific activity was measured directly, obviated the need for determining exchange constants between brain and plasma. Pelligrino et al. (1987) found the rate of DG-6-P accumulation decreased with time in a manner that could not be explained by the operational equation of the DG method. The discrepancy was significant by 10–20 min, and the data could be rectified by the introduction of a single loss constant operating throughout the entire experimental period (Table 2). Pelligrino et al. (1987) concluded "that when using the DG method in the goat, DG-6-P dephosphorylation cannot be ignored when employ-

[2] After a single intravenous injection, arteriovenous differences of DG across cat brain (Sokoloff et al., 1977) and rat brain (Sacks et al., 1983) were found to be positive, indicating uptake, but after 5–10 min they became negative and remained so thereafter, indicating a continuous loss of label from brain. Sacks et al. (1983) interpreted these results to indicate that DG-6-P formed at early times was hydrolyzed and the label released as DG. Conversely, Nelson et al. (1986*a*) maintained that the results are the behavior predicted by the DG model. Using established rate constants of DG exchange (which they indicate elsewhere in their paper may be inaccurate), they show curves predicting negative arteriovenous differences for DG across the whole brain, concurrent with positive arteriovenous differences for glucose. Their calculations seem to conflict with data in the article by Sokoloff et al. (1977, Fig. 5) which predict the total radioactivity in brain—[^{14}C]DG plus [^{14}C]DG-6-P—to rise continuously. Since net accumulation can occur only when the arteriovenous difference is positive, the occurrence of substantial negative arteriovenous differences beginning between 5 and 10 min stands in contradiction.

Nelson et al. (1986*a*) also presented representative arteriovenous differences of [^{14}C]DG measured in individual animals in which arterial concentrations were kept constant. There was no apparent tendency for the arteriovenous differences to lessen with time. The authors argued that these data are evidence for no significant DG-6-Pase activity in brain. Since the data shown were from single selected animals, statistical analyses are, of course, impossible. It may be noted that earlier data were not so clear-cut. Close examination of Fig. 4A from Sokoloff et al. (1977) shows decreasing values for arteriovenous differences across rat brain as time passed, and Fig. 4B in the same article depicts increasingly lower values for the "lumped constant." The earlier data, therefore, could be interpreted as evidence for DG-6-P loss and as evidence that the value for the "lumped constant," determined by arteriovenous differences, would decrease with time. The discrepancies between the former and latter studies remain to be clarified.

ing more than 10-min evaluation periods." As mentioned above, other investigators have likewise found it necessary to incorporate a term for loss of FDG-6-P into calculations made during studies in humans (Phelps et al., 1979; Kato et al., 1984) and dogs (Kato et al., 1984). These rate constants are applied from the beginning of the experiment, apparently being fit without a time lag (Table 2).

The above data derived from a variety of species provide evidence that DG-6-P loss begins shortly after it is formed and is continuous thereafter. This interpretation helps to explain a phenomenon that has now been observed in several laboratories. Specifically, calculated values of CMRglc are high at early times and decrease thereafter, whereas the uncorrected DG method predicts that calculated CMRglc values should be constant (at least for 45 min). The data of Nelson et al. (1986*a*) show the effect quite clearly (reproduced in Fig. 3). The results were attributed as being due to "inaccuracies in the values of rate constants which could not possibly be accurate for the brain as a whole." (The rate constants of efflux and phosphorylation of DG would have to be raised to infinity to make their measured and their theoretical curves agree.) This argument does not hold up well because the same trend (calculated CMRglc decreasing as time passes) is seen when CMRglc is calculated from actual tissue DG content, thereby avoiding assumptions about the rate constants (Fig. 3B). These results are what would be predicted by the continuous dephosphorylation of DG-6-P. In conclusion, the data of Nelson et al. (1986*a*) provide further evidence that DG-6-P loss begins at an early time. The hypothesis that compartmentation provides a prolonged period of substrate sequestration before the phosphatase can act is not well supported by the available data.

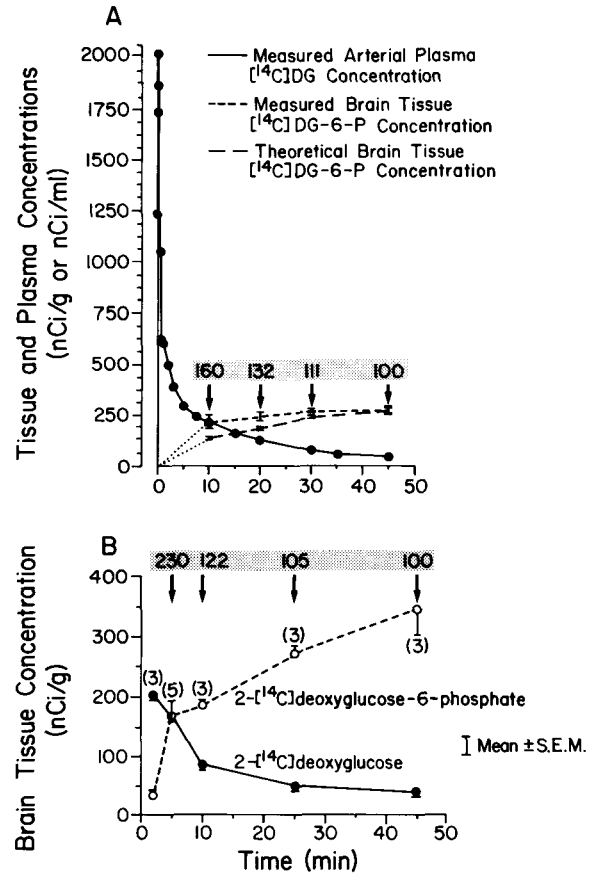

FIG. 3. Apparent CMRglc is not closely predicted by the DG equation. **A:** Figure 2 redrawn from Nelson et al. (1986a) with the apparent CMRglc indicated at various times as a percentage of the 45-min value. The apparent CMRglc was calculated directly from the ratio of the observed to expected DG-6-P values calculated by Nelson et al. (1986a). **B:** Figure 1A redrawn from Nelson et al. (1986a) with the apparent CMRglc, as a percentage of the 45-min value, calculated directly from the integral of brain DG. In this example no assumptions about exchange rates are made.

IS LOSS OF DG-6-P HOMOGENEOUS THROUGHOUT BRAIN?

There are data indicating that G-6-Pase activity is not homogeneously distributed at either the cellular or regional level (Broadwell and Cataldo 1983, 1984; Cataldo and Broadwell, 1986). Duncan et al. (1986) compared the accumulation of ^{14}C from [1-^{14}C]glucose and [^{14}C]DG at the microscopic level. Label accumulated more rapidly from [1-^{14}C]glucose, but most importantly they found that some areas in brain showed a different ratio of [^{14}C]DG to [1-^{14}C]glucose. They suggested that the results could be explained if [^{14}C]DG-6-P was being selectively hydrolyzed. Hawkins et al. (1988) found that regional differences existed; the relative rates of DG-6-P loss were as follows: telencephalon < diencephalon = mesencephalon < metencephalon < myelencephalon. The occurrence of regional differences in the rate of DG-6-P loss raises the possibility that different correction factors may be required for each anatomic structure (or even in different cells). Clearly this is an area where further experiments should be undertaken.

IS LOSS OF DG-6-P CONSTANT OR IS IT REGULATED?

If phosphatase activity is constant in a region it is a relatively simple matter to correct for its effect mathematically once the activity in vivo has been characterized. If, on the other hand, phosphatase activity changes according to physiological or pathological conditions then the modeling of this process becomes more complex. This particular aspect has not received much attention, but evidence suggests that phosphatase activity can be stimulated. Anchors and Karnovsky (1975) demonstrated that G-6-Pase is activated during slow-wave sleep. In other experiments Karnovsky et al. (1980) showed that an accelerated loss of DG-6-P occurs in sleeping rats. In these experiments a group of sleep-deprived rats was injected

with DG. At 45 min half the rats were allowed to sleep for 15 min while the remainder were kept awake. At 60 min all rats were killed and the brains analyzed for DG-6-P. The brains of sleeping rats lost 30% of DG-6-P in only 15 min. Pelligrino et al. (1987) also found evidence of G-6-Pase regulation. In goats anesthetized with pentobarbital the glucose phosphorylation rate was underestimated by 46.5% compared to 23.1% with N_2O anesthesia because the dephosphorylation to phosphorylation ratio was increased substantially. The experiments of Karnovsky et al. and Pelligrino et al. suggest that G-6-Pase is a regulated enzyme, and underscore the need to investigate further the effects of changes in physiological and pathological conditions on DG-6-P loss in brain.

DISCUSSION

A necessary part in the computation of CMRglc from the accumulation of DG-6-P is the proportionality factor known as the "lumped constant." This factor takes into account the different kinetics of glucose and deoxyglucose entry into brain and the different kinetics of phosphorylation. Some recent work indicates that the "lumped constant" originally determined for use in the DG method in rats may be in error because G-6-Pase activity was not appreciated. The "lumped constant" is the ratio of cerebral extraction of DG to glucose from blood when both are in steady states. To determine its value, a programmed infusion designed to raise and maintain arterial [^{14}C]DG concentration can be used and arteriovenous differences of both [^{14}C]DG and glucose measured after a steady state is reached. If, however, G-6-Pase activity is present, hydrolysis of DG-6-P may occur with a concomitant return of DG to the plasma. Since the rate of hydrolysis is proportional to the amount of DG-6-P present in the brain, and since DG-6-P will continue to accumulate during the infusion, the effect will be to lower the arteriovenous difference as time passes and thereby decrease the estimate of the "lumped constant" (Sacks et al., 1983; Huang and Veech, 1985). Hargreaves et al. (1986) reevaluated the "lumped constant" in rat brain by an independent method that precluded significant DG-6-P loss. The value they determined was 0.65, about 35% higher than that estimated (0.48) from the relative extractions of analog and glucose during a 45-min programmed infusion (Sokoloff et al., 1977). Hargreaves et al. suggest the discrepancy could be accounted for by phosphatase activity acting over the 45 min period at a rate of 1%/min, thereby resulting in lower net accumulation of DG-6-P. If this explanation is correct then estimates of the "lumped constant" by the arteriovenous difference method are critically dependent on time; the values must decrease as time passes.

Nelson et al. (1986*a*) have noted a discrepancy between the theoretical accumulation of DG-6-P by

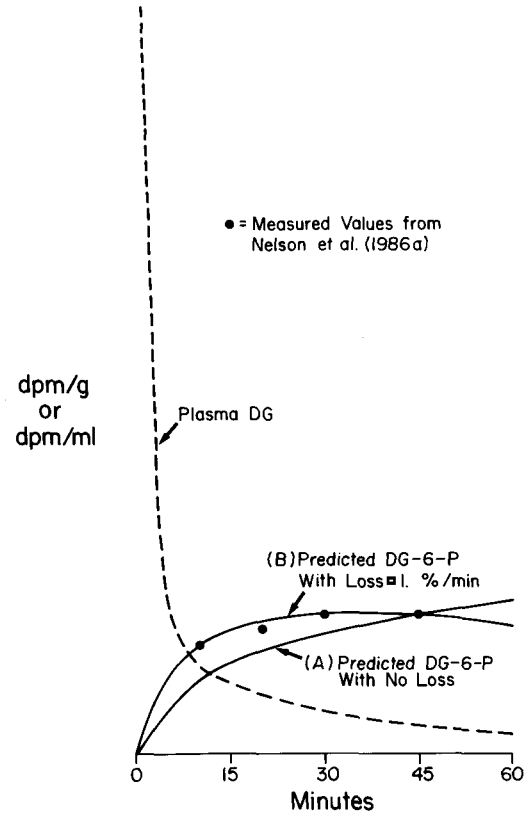

FIG. 4. Two distinct models. The accumulation of DG-6-P predicted by the operational equation of the DG method is shown in curve A calculated as described by Sokoloff et al. (1977). Curve B shows the predicted accumulation of DG-6-P under the same conditions, but where the "lumped constant" was 0.65 and loss of DG-6-P assumed to occur continuously at a rate of 1%/min (see Hargreaves et al., 1986). The filled circles are experimental values of DG-6-P, measured by Nelson et al. (1986*a*), normalized to the maximal value at 45 min. The experimental data appear to be better fit at all times by a model including a correction for dephosphorylation.

brain and the DG-6-P that was actually measured (Fig. 3A). However, by using the "lumped constant" determined by Hargreaves et al. (1986) and taking the rate of loss to be 1%/min, a theoretical curve may be calculated that passes through the experimental points much more closely (Fig. 4). These results suggest that a revision of current estimates of the "lumped constant" may be necessary as well as to take into account dephosphorylation.

CONCLUDING REMARKS

Although DG was originally chosen to measure CMRglc because it was believed that its metabolite DG-6-P was trapped irreversibly in brain cells, this view is difficult to maintain. The evidence reviewed above shows that DG-6-P loss can be significant and that it is most likely that it begins soon after DG administration. The important questions remaining are: how much is lost, from where, how much the loss is altered by different physiological and pathological

conditions, and what the impact is on the analysis of DG data. A series of carefully designed experiments are required to answer these questions satisfactorily.

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REFERENCES

Anchors J. M. and Karnovsky M. W. (1975) Purification of cerebral glucose-6-phosphatase—an enzyme involved in sleep. *J. Biol. Chem.* **250,** 6408–6416.

Anchors J. M., Hagerty D. F., and Karnovsky M. L. (1977) Cerebral glucose-6-phosphatase and the movement of 2-deoxy-D-glucose across cell membranes. *J. Biol. Chem.* **252,** 7035–7041.

Arion W. J., Wallin B. K., Lange A. J., and Ballas L. M. (1975) On the involvement of a glucose-6-phosphate transport system in the function of microsomal glucose-6-phosphatase. *Mol. Cell Biochem.* **6,** 75–83.

Bachelard H. S. (1985) Review of *Metabolic Probes of Central Nervous System Activity in Experimental Animals and Man. J. Neurochem.* **44,** 1963–1965.

Baginski E. S., Foa P. P., and Zak B. (1967) Determination of rat liver microsomal glucose-6-phosphatase activity. *Anal. Biochem.* **21,** 201–207.

Ballas L. M. and Arion W. J. (1977) Measurement of glucose-6-phosphate penetration into liver microsomes—confirmation of substrate transport in the glucose-6-phosphatase system. *J. Biol. Chem.* **252,** 8512–8518.

Broadwell R. D. and Cataldo A. M. (1983) The neuronal endoplasmic reticulum: its cytochemistry and contribution to the endomembrane system. I. Cell bodies and dendrites. *J. Histochem. Cytochem.* **31,** 1077–1088.

Broadwell R. D. and Cataldo A. M. (1984) The neuronal endoplasmic reticulum: its cytochemistry and contribution to the endomembrane system. II. Axons and terminals. *J. Comp. Neurol.* **230,** 231–248.

Cataldo A. M. and Broadwell R. D. (1986) Cytochemical identification of cerebral glycogen and glucose-6-phosphatase activity under normal and experimental conditions: 1. Neurons and glia. *J. Electronmicosc. Tech.* **3,** 413–437.

Colilla W., Jorgensen R. A., and Nordlie R. C. (1975) Mammalian carbamyl phosphate:glucose phosphotransferase and glucose-6-phosphate phosphohydrolase: extended tissue distribution. *Biochim. Biophys. Acta* **377,** 117–125.

Cunningham V. J. and Cremer J. E. (1985) Current assumptions behind the use of PET scanning for measuring glucose utilization in brain. *Trends Neurosci.* **8,** 96–99.

Deuel R. K., Yue G. M., Sherman W. R., Schickner D. J., and Ackerman J. J. H. (1985) Monitoring the time course of cerebral deoxyglucose metabolism by ^{31}P nuclear magnetic resonance spectroscopy. *Science* **228,** 1329–1331.

Duncan G. E., Pilgrim C., Stumpf W. E., McCown T. J., Breese G. R., and Mueller R. A. (1986) High resolution autoradiographic determination of a topographic distribution of radioactivity in the hippocampal formation after injection of [^{14}C]glucose or 2-deoxy[^{14}C]glucose. *Neuroscience* **17,** 99–106.

Fishman R. S. and Karnovsky M. L. (1986) Apparent absence of a translocase in the cerebral glucose-6-phosphatase system. *J. Neurochem.* **46,** 371–378.

Fox J. L. (1984) PET scan controversy aired. *Science* **224,** 143–146.

Hargreaves R. J., Planas A. M., Cremer J. E., and Cunningham J. (1986) Studies on the relationship between cerebral glucose transport and phosphorylation using 2-deoxyglucose. *J. Cereb. Blood Flow Metab.* **6,** 708–716.

Hawkins R. A. and Miller A. L. (1978) Loss of radioactive 2-deoxy-D-glucose-6-phosphate from brains of conscious rats: implications for quantitative autoradiographic determination of regional glucose utilization. *Neuroscience* **3,** 251–258.

Hawkins R. A., Phelps M. E., Huang S. C., and Kuhl D. E. (1981) Effect of ischemia on quantification of local cerebral glucose metabolic rate in man. *J. Cereb. Blood Flow Metab.* **1,** 37–51.

Hawkins R. A., Mans A. M., Davis D. W., and DeJoseph M. R. (1988) Comparison of ^{14}C-glucose and ^{14}C-deoxyglucose as tracers of brain glucose use. *Am. J. Physiol.* (in press).

Hers H. G. (1957) *Le Metabolisme du Fructose,* p. 102. Editians Arscia, Bruxelles.

Huang M. T. and Veech R. L. (1985) Metabolic fluxes between [^{14}C]2-deoxy-D-glucose and [^{14}C]2-deoxy-D-glucose-6-phosphate in brain *in vivo. J. Neurochem.* **44,** 567–573.

Huang S.-C., Phelps M. E., Hoffman E. J., Sidevis K., Seilin C. J., and Kuhl D. E. (1980) Noninvasive determination of local cerebral metabolic rate of glucose in man. *Am. J. Physiol.* **238,** E69–E82.

Karnovsky M. L., Burrows B. L., and Zoccoli M. A. (1980) Cerebral glucose-6-phosphatase and the movement of 2-deoxyglucose during slow wave sleep, in *Cerebral Metabolism and Neural Function* (Passoneau J. V., Hawkins R. A., Lust W. D., and Welsh F. A., eds), pp. 359–366. Williams & Wilkins, Baltimore.

Karnovsky M. L., Anchors J. M., and Zoccoli M. A. (1982) Glucose-6-phosphatase from cerebrum, in *Methods in Enzymology, Vol. 90* (Wood W. A., ed), pp. 396–402. Academic Press, New York.

Kato A., Diksic M., Yamamoto L., Strother S. C., and Feindel W. (1984) An improved approach for measurement of regional cerebral rate constants in the deoxyglucose method with positron emission tomography. *J. Cereb. Blood Flow Metab.* **4,** 555–563.

Leskes A., Siekevitz P., and Palade G. E. (1971) Differentiation of endoplasmic reticulum in hepatocytes I. Glucose-6-phosphatase distribution *in situ. J. Cell. Biol.* **49,** 264–287.

Nelson T., Lucignani G., Goochee J., Crane A. M., and Sokoloff L. (1986*a*) Invalidity of criticism of the deoxyglucose method based on alleged glucose-6-phosphatase activity in brain. *J. Neurochem.* **46,** 905–919.

Nelson T., Lucignani G., and Sokoloff L. (1986*b*) Measurement of brain deoxyglucose metabolism by NMR. *Science* **232,** 776–777.

Nordlie R. C. (1971) Glucose-6-phosphatase, hydrolytic and synthetic activities, in *The Enzymes, Vol. 4* (Boyer P. E., ed), pp. 543–610. Academic Press, New York.

Nordlie R. C. (1975) Metabolic regulation by multifunctional glucose-6-phosphatase. *Curr. Top. Cell. Regul.* **8,** 33–117.

Nordlie R. C. and Arion W. J. (1965) Liver microsomal glucose 6-phosphatase, inorganic pyrophosphatase and pyrophosphate-glucose transferase. *J. Biol. Chem.* **240,** 2155–2164.

Pelligrino D. A., Miletich D. J., and Albrecht R. S. (1987) The time course of radio-labeled 2-deoxyglucose phosphate turnover in the cerebral cortex of the goat. *Am. J. Physiol.* **252,** R276–R283.

Phelps M. E., Huang S. C., Hoffman E. J., Selin C., Sokoloff L., and Kuhl D. E. (1979) Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18)2-fluoro-2-deoxy-D-glucose: validation of method. *Ann. Neurol.* **6,** 371–388.

Prasannan K. G. and Subrahamanyam K. (1968) Effect of insulin on the synthesis of glycogen in cerebral cortical slices of alloxan diabetic rats. *Endocrinology* **82,** 1–6.

Rosen S. I. (1970) Glucose-6-phosphate hydrolysing activity in the cerebellum of the mouse, rat, hamster and marmoset. *Acta Histochem.* **36,** 44–53.

Sacks W., Sacks S., and Fleischer A. (1983) A comparison of the cerebral uptake and metabolism of labeled glucose and deoxyglucose *in vivo* in rats. *Neurochem. Res.* **8,** 661–685.

Sokoloff L. (1982) The radioactive deoxyglucose method: theory,

procedure, and applications for the measurement of local glucose utilization, in *Advances in Neurochemistry* (Agranoff B. W. and Aprison N. H., eds), pp. 1–82. Plenum Press, New York.

Sokoloff L., Reivich M., Kennedy C., Des Rosiers M. H., Patlak C. S., Pettigrew K. D., Sakurada O., and Shinohara M. (1977) The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J. Neurochem.* **28,** 897–916.

Stephens H. R. and Sandborn E. B. (1976) Cytochemical localization of glucose-6-phosphatase activity in the central nervous system of the rat. *Brain Res.* **113,** 127–146.

Welch G. R. (1977) On the role of organized multienzyme systems in cellular metabolism: a general synthesis. *Prog. Biophys. Mol. Biol.* **32,** 103–191.